REMARKS

Reconsideration of the rejections set forth in the Office action mailed May 11, 2005 is respectfully requested. Claims 1, 4-7 and 11-24 are pending in the application.

The applicants thank the Examiner for the opportunity to discuss the invention and the cited art on February 2, 2006. An Interview Summary Record is enclosed summarizing the content of this discussion.

I. Amendments

No amendments are made with this response.

II. The Invention

The applicant's invention, as embodied in independent claim 1, is directed to a method of electrophoretically injecting a sample containing multiple charged components into a microfluidic device, and electrophoretically separating the components. The method as claimed employs a two-electrode injection step and a two-electrode separation step, and combines this voltage control scheme with ITP stacking, by supplying in either the sample solution or the buffer electrolyte solution a high mobility ion which is present at a higher concentration than the sample ions. This method produces detectable peaks of high sharpness and resolution, while maintaining high sample volume, thus producing high peak intensities as well.

As discussed further below, cited reference Manz *et al.* describes a "pushback" strategy (which is referred to in the applicants' specification as "pullback"), and cited reference Ramsey *et al.* describes a "pinch" injection strategy. Both of these schemes employ simultaneous control of at least three electrodes. They have been shown to increase sample resolution and signal-to-noise ratio, but usually at the expense of signal intensity, due to loss of sample.

The applicants have found that the use of transient isotachophoretic (ITP) stacking, which can be implemented as recited in steps (a)-(c) of claim 1, employed in combination with a two-electrode injection and two-electrode separation strategy (i.e. sample and drain voltage on for injection, followed by upstream and downstream voltage on for separation), gave significantly better peak sharpness and intensity than a "pinch/pullback" injection scheme, when the latter was done either with or without such ITP stacking. This is illustrated in Fig. 5C, which superimposes

Figs. 5A and 5B. As shown in the Figures, while the "pinch/pullback" strategy gives a sharp and well resolved signal peak, its intensity is greatly reduced relative to the "floating" strategy, presumably due to sample loss.

The top two traces in Fig. 6A (which are offset for clarity) compare the "pushback" strategy described in Manz (labeled "FL INJ, PB SEP"; no pinching, no stacking) with the method of the invention (labeled "FL + STACK"; i.e. two electrode injection and two electrode separation, with ITP stacking). These results show that the method of the invention clearly provides greater peak sharpness and much greater peak intensity, with near-equivalent resolution (i.e. peak separation).

II. Rejections under 35 U.S.C. §103

The rejection of claims 1, 4-7 and 14-24 under 35 U.S.C. §103(a), as being unpatentable over Manz *et al.* (U.S. Patent No. 6,280,589) in view of Krivankova *et al.* (*J. Chromatography B* **689**:13-34, 1997), has been maintained. The rejections are respectfully traversed in light of the following remarks.

A. The Cited Art

Manz *et al.* describes, in the Background section and at column 5, lines 31-58, a prior art two-electrode sample injection process, in which sample is introduced into a main channel by applying an appropriate voltage to each of two channels intersecting the main channel, one of which contains sample. A problem with this process, as described by Manz (e.g. at column 1, lines 51-67 and column 5, lines 59-63), is leakage of residual sample from the side channel(s) into the main channel after sample introduction is formally completed.

To address this problem, Manz *et al.* describes the "pushback" (aka "pullback") strategy, where voltage is applied to induce flow back into the side channels, to prevent the above-described leakage (column 5, line 63 to column 6, line 5; column 6, lines 20-39).

As described in the applicants' specification, this strategy improves resolution and signal-to-noise ratio, but it also leads to loss of sample volume.

Krivankova *et al.* describes the phenomenon of "sample induced stacking" or "sample induced transient ITP". It illustrates a coupled system where transition occurs from an ITP

stacking mode to a CZE mode. The systems described employ capillaries, not microfluidic channels.

C. Analysis

C1. No Motivation to Combine References

The applicants contend that one skilled in the art would not have been motivated to combine the teachings of Krivankova *et al.* with those of Manz *et al.* The two references describe systems having significant structural differences and uses, and are directed to different perceived problems and benefits.

Manz describes a system in which sample is electrokinetically introduced into a main separation channel region between side channels in a "double T" configuration, as illustrated in Fig. 3 of the patent. In using this "valveless device", "injection of the sample plug into the electrolyte channel is accomplished electro-kinetically by applying an electric field across the supply and drain channels" (column 2, lines 60-63). In order to avoid the resulting problem of bias of sample composition, due to different electrophoretic mobilities of the components (noted at column 2, lines 8-12), the injection is carried out "for a time at least long enough that the sample component having the lowest electrophoretic mobility is contained within the geometrically defined volume" (column 2, lines 63-66).

It can be seen, therefore, that this system employs fairly small sample volumes, since a representative aliquot of the original sample must be electrokinetically introduced into the "geometrically defined volume" between the two side channels.

Krivankova *et al.* describes the usefulness of preceding capillary zone electrophoresis (CZE) with a preconcentration step employing capillary isotachophoresis (ITP). The advantage of this combination, as described, for example, at page 15 of the reference, is that, due to the preconcentrating effect of ITP, "a larger volume of a more diluted sample could be injected", allowing detection of analytes present at low concentration in a sample. In order to handle the "relatively large volume of a sample", a "capillary of wide diameter equipped with a sample valve is recommended" for injection (page 18, second column). The ITP and CZE capillaries are "interconnected via a bifurcation block which ensures that only a proper part of the ITP zone stack

is transferred into the CZE step", concurrent with "removal of the ballast from the sample" (page 19).

Accordingly, Krivankova *et al.* teaches the use of a sample valve, for injecting a large volume of sample, and a "bifurcation block" for transferring a small portion of this volume, following ITP, to the separation zone.

This injection process clearly differs significantly from that shown in Manz. There is no provision in the device of Manz to allow for these large sample volumes, or for "bifurcation", to remove "ballast" sample volume, as taught in Krivankova *et al.*

The references are also directed towards different perceived problems and solutions. In Krivankova *et al.*, as noted above, the problem is the analysis of samples having analytes present in low concentration, such that large sample volumes are required for accurate detection (as described at page 15, second column). This is addressed by pre-concentrating the sample by ITP and diverting the "ballast" solution prior to CZE separation.

Manz, on the other hand, is directed to solving the problem of leakage of residual sample from the side channel(s) of their "valveless injection device" into the main separation channel after sample introduction is formally completed (as described at column 1, lines 51-67 and the paragraph bridging columns 5-6). This problem is addressed by applying voltage, during separation, to induce flow back into the side channels, a strategy referred to as "pushback" (aka "pullback").

There is no suggestion in either of the references that this problem could be addressed by ITP stacking. In fact, in the reference cited in the Background of Manz *et al.* (Verheggen *et al.*, *Journal of Chromatography*, **452**, 612-622, 1988) this "leakage" problem is described as applicable to ITP separations as well as to CZE separations. See, for example, the first paragraph on page 617 of the article (Verheggen *et al.*, provided via facsimile with the Interview Request Form on January 25, 2006).

The applicants also disagree with the Examiner's contention that the passage at column 1, lines 48-51 of Manz "show that Manz et al. viewed sample preconcentration/stacking as an advantage" (Office Action dated May 11, 2005, page 13, lines 1-3). The statement relied on by the Examiner is, in its context, ambiguous at best:

> An advantage of the double T shape sampling device, as is also obtained with the use of injection valves, is the concentration effect of dilute sample ionic species.

It cannot be determined from the context of this statement in Manz *et al.* how the "double T shape sampling device", in common with "injection valves", would provide a "concentration effect of dilute sample ionic species". Certainly, one reading the patent would have no reason to conclude that it referred to ITP stacking.

Moreover, by consulting the reference to which Manz *et al.* refer in their initial description of the "double T shape sampling device" (Verheggen *et al.*, provided via facsimile with the Interview Request Form on January 25, 2006), one finds a very similarly worded statement (emphasis added in all citations):

> In this paper a simple sampling device for capillary ITP and CZE is described, whereby the sample solution is introduced directly into part of the capillary tube by means of two feeders, perpendicular to the capillary tube. The sample can be introduced *without mixing with the background electrolyte*. **An advantage, as obtained with the use of a valve, is the concentration effect of dilute sample ionic species.** (page 616, first full paragraph)

The reference further states the following, in the Introduction:

> The best way of sampling in CZE is to introduce a representative aliquot of a sample, concentrated as much as possible in a narrow band and *without mixing with the background electrolyte*. ...
>
> A sample valve is, in fact, the most suitable sampling method for CZE. With a sample valve a known volume of a representative aliquot of the sample can be introduced *without mixing with the background electrolyte*. (page 615, first and second paragraphs)

and in the concluding Discussion:

> Using the new sampling device... Compared with injection by a syringe this method has the advantage that there is *no mixing with other electrolyte solutions*, in either the ITP or the CZE mode. (page 622, first paragraph)

It is apparent from these passages that the advantageous "concentration effect" in fact refers to the fact that there is no "mixing with the background electrolyte" required for either the "new sampling device" or an injection valve. This "effect" has nothing to do with ITP or CZE per se.

C2. Unsuggested Benefits

One way for a patent applicant to rebut a prima facie case of obviousness is to make a showing of "unexpected results," i.e. to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected. *In re Soni*, 54 F.3d 746, 34 USPQ2d 1684 (Fed. Cir. 1995).

As described in Section I above, the method of the claims gave significantly better peak sharpness and intensity than a "pinch/pullback" injection scheme, when the latter was done either with or without such ITP stacking.

There is no suggestion in either of the above-cited references that the use of ITP stacking in a two-electrode ("floating") injection/separation scheme would address the "leakage" problem noted by Manz *et al.*, in a microchannel device, providing high peak sharpness and good resolution, without the concomitant loss of signal intensity that results from the Manz method (described in applicants' specification as "pullback").

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

III. Further Rejections under 35 U.S.C. §103(a)

Dependent claims 11-12 were rejected under 35 U.S.C. §103(a) as being unpatentable over Manz *et al.* (U.S. Patent No. 6,280,589) in view of Krivankova *et al.* (*J. Chrom. B* **689**:13-34 (1997)), as applied to parent claim 1 above, and further in view of Ramsey (U.S. Patent No. 6,342,142). The rejections are respectfully traversed in light of the following remarks.

Dependent claims 11-12 are directed to types of analytes that may be used in the method of claim 1.

For the reasons discussed above, Manz *et al.* in view of Krivankova *et al.* does not teach or suggest the method of claim 1, or the benefits thereof. With respect to the subject matter of independent claim 1, Ramsey *et al.* does not make up for the deficiencies of this combination of references. For example, at column 5, line to 52 to column 6, line 13, Ramsey *et al.* describes the benefits of "pinched" injection over "floating" injection. The reference does not suggest the

approach taken by the applicants, where ITP stacking is used in combination with "floating" injection and separation, and provides superior results to the "pinch/pullback" combination.

Accordingly, claim 1 and its dependent claims are nonobvious over this combination of references.

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

V. Further Rejections under 35 U.S.C. §103(a)

Claims 1 and 13 were rejected under 35 U.S.C. §103(a) as being unpatentable over Fuchs *et al.* (U.S. Patent No. 5,630,924) in view of Manz *et al.* and Krivankova *et al.*, as presented above. The rejections are respectfully traversed in light of the following remarks.

A. The Cited Art

Manz *et al.* and Krivankova *et al.* are discussed above.

Fuchs *et al.* is directed to an assay method in which an analyte binds with a first binding partner, which is labeled, and with a second binding partner, which is modified to impart a significant charge. The binding partners are typically modified antibodies. The method is stated to improve over previous assays which did not employ the second binding partner, and where electrophoretic separation of unbound analyte from bound complex was less effective. (See, for example, column 1, lines 45-59 and column 2, lines 44-58.)

While there is a general description of electrophoretic mobility of species in Fuchs (column 16, lines 15-52), there is no description of particular voltage-controlled injection schemes, and, in fact, injection may be carried out using a pump rather than by voltage control (column 21, lines 26-38).

One stage of the assay in Fuchs involves mixing of the analyte and binding partners. One way of mixing these components is noted at column 23, lines 51-53, where "the elements of the mixture were concentrated in an electric field using a technique such as isoelectric focusing or isotachophoresis". There is no other reference to isotachophoresis (ITP) in the patent.

B. Analysis

Fuchs *et al.* does not suggest the advantages of ITP in a separation method. As stated above, the only reference to ITP in Fuchs is as one way of mixing the assay components: "the elements of the mixture were concentrated in an electric field using a technique such as isoelectric focusing or isotachophoresis". Presumably, the assay components are "concentrated" to facilitate reaction (binding) before the separation and detection of bound complex is carried out. Mixing of the components is further described at column 14, line 34-41:

> In one embodiment, first binding partner and second binding partner are combined with a sample to produce a mixture in which, if analyte is present, a three-membered complex forms. As used herein, the term "combine" is intended to mean any process by which multiple components are brought together for subsequent interaction at the molecular level.

The sole teaching of this reference with respect to ITP, then, is that it can be can be used as one way to combine assay components in a channel in high concentration, to facilitate reaction of the components with each other. This would not be perceived as a useful benefit per se in a process in which the ultimate goal is to separate all of the components from each other. There is no suggestion that the use of ITP stacking would produce any particular advantage with respect to separation.

In particular, none of the references, alone or in combination, suggest the benefits of ITP stacking discovered by the applicant, where the use of sample stacking in combination with a simple two-electrode injection scheme in a microchannel device produced separation results superior to the "pullback" and "pinching" strategies described in the prior art.

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

VI. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4403.

Respectfully submitted,

Date: Feb 13, 2006

LeeAnn Gorthey
Registration No. 37,337

Correspondence Address:
PAYOR NUMBER 22918
PHONE: (650) 838-4403
FAX: (650) 838-4350